

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

The Change of Oxidation-Reduction Potentials of Water-logged Soils. II.—Lateritic Soils and Sandstone-Shale Soils. (J. Agr. Chem. Soc. Japan, **12**, pp. 1141~1151, 1936): By Kisaburo SHIBUYA, Hideaki SAEKI and Kenhan RYU. (Laboratory of Soils and Fertilizers, Taihoku Imperial University, Received September 24, 1936.)

In the previous paper (J. Agr. Chem. Soc. Japan, **12**, 62, 1936), the authors published a report concerning general investigation on the change of oxidation-reduction potentials of soils which were placed under water-logged condition for 100 days. It dealt with, in the present paper, the result of further investigation on the same subject, in special reference to factors affect the change of oxidation-reduction potentials of water-logged soils. The soil sample used for the study was a lateritic soil at Heichin and a sand-stone-shale soil of a rice field in Taihoku. The former contains a significant amount of iron oxide and a poor quantity of organic matter, while the latter is rich in humus to more or less extent.

The following experiment was carried out in this investigation, viz.,

- 1) Changes of oxidation-reduction potentials of the soil which was varied his pH values by HCl or Ca(OH)₂.
- 2) Changes of oxidation-reduction potentials, reaction and FeO content of the soil which was placed under water-logged condition after varying his pH.
- 3) Changes of oxidation-reduction potentials and reaction of the soil which was in water-logged condition after addition of HgCl₂-solution, in order to prevent microbiological activities.
- 4) Changes of oxidation-reduction potentials and reaction of the soil which was in water-logged condition after mixing Fe₂O₃.

5) Changes of oxidation-reduction potentials, reaction and FeO quantity of iron oxide which was similarly treated to the soil in water-logged condition.

6) Changes of oxidation-reduction potentials and reaction of the soil which were in water-logged condition after addition of mannite to 20 per cent of soils.

7) Changes of oxidation-reduction potentials and reaction of water extracts of the soil in a series. The water extract was mixed with mannite in proportion as it was applied to the soil and was kept in a number of glass tubes, for about 100 days, without contact with atmosphere by means of forming a film of liquid paraffin on the surface.

8) Changes of oxidation-reduction potentials and reaction of water extracts of the soil in another series. Those were prepared and tested by the same method as described in 7), except that they were added $HgCl_2$ -solution to extent of 1000 p. p. m..

The result of this investigation is to be summarized as it follows:

1) Oxidation-reduction potentials of the soil which was varied his pH values by addition of acid or alkaline solution, varied themselves in an inverse relation to pH values of the soil. Potentials of the strongly acidified soil, in comparison with chinhydron line, were lower than those of chinhydron at the same pH value, while potentials of the less acidified soil were higher.

2) Oxidation-reduction potentials of the soil which was varied his pH values, respectively, to 4.0 and 7.0 by acid or alkaline solution, markedly sunk down within the first one week, under water-logged condition. Those depression, thereafter, slowly and insignificantly continued. Ferrous oxide determined in the soil gradually increased, in accordance with advancement of the water-logging period. It was noticed that oxidation-reduction potentials of the soil which was artificially varied his reaction, changed themselves by the similar behavior to the original soil under water-logged condition. The reaction of soil of pH 4.0, accordingly, approached to neutral point and that of soil of pH 7.0 returned to the same point, although it slightly went down to the acid side at the beginning.

3) The soil which was mixed pure ferric oxide, displayed an increasing depression of his oxidation-reduction potentials under water-logged condition. It obviously depended on the added ferric oxide.

4) Oxydation-reduction potentials of the soil which was added $HgCl_2$ -solution to extent of 1000 p. p. m. and was placed under water-logged condition for about 100 days, depressed themselves to a significant degree. It was, especially, more significant in the lateritic soil whose Fe_2O_3 content was greater. Nature of the potential depression was, however, a little different from that of the soil without addition of $HgCl_2$. That was nearly to be

traced a straight line, revealing no significant depression within the first one week. It was rather similar to the potential change of pure iron sesqui-oxide. It should be said, therefore, that the potential change of the soil which was added $HgCl_2$ -solution, was, partly at least, affected by sesquioxide of iron under water-logged condition.

5) Mannite was added, on the other hand, to the soil as a nutrient of micro-organisms, in order to exert their activities. Oxidation-reduction potentials of such soil markedly sunk down under water-logged condition, as they displayed by the original soil, noticeably depressing themselves in the earlier period of water logging.

6) Potentials of water extracts which were obtained from the same soil samples prepared as above described, similarly depressed themselves at the beginning of the period.

7) When the water extract was added mannite and $HgCl_2$ -solution (1000 p. p. m.) it showed not noticeable changes.

By those experimental consequences, in general, it should be concluded that oxidation-reduction potentials of soils depress themselves, on response to chemical processes of micro-organisms on the potential reveals in earlier but that of inorganic soil materials takes place in a little later period of water logging.

It is, furthermore, noticed that the potential of water-logged soils again rise up to a small extent at 4~5 weeks after submerging the soil. This is to be attributed to formation of organic acids in the solution depending on decomposition of soil organic matter by micro-organisms. It shows that the acid formation slightly exceeded over reducing action between the potential change in this period.

The Cause of Decrease of Ether Extract in Fish Meal during Storage. (pp. 1152~1162): By Kôkichi ÔSHIMA and Tatsurô SUGAWARA. (The Chemical Laboratory of Hakodate College of Fisheries, Japan. Received October 3, 1936.)

It has been observed that the content of ether extract (crude fat) in fish meal occasionally decreased during storage. Yet no one has determined under what conditions the decrease took place.

The authors have made repeated experiments in the following manner:—

1. For this purpose, as the sample, fresh sardine meal made by machine with steam dryer was used. The ether extract content was about 10% at the beginning.

2. The sardine meal was stored in bags or bottles with or without exposure to air or sunlight for about one year. At intervals of 2 to 8 weeks,

the content of ether extract and iodine value were determined.

3. The sardine meal was heated to 80~100°C and kept at that temperature for a few days and the decrease of the ether extract was determined.

4. Sardine and herring oil with oil free fish meal, oil free cotton or filter paper were kept at 100°C for a few days and the change of total weight and ether extract content were measured.

5. The ether extracted sardine meal was hydrolyzed with HCl or pepsin and the ether extract content was determined.

6. Ether, benzol and acetone were compared to determine their ability to extract the oxydized fish oil.

7. Many samples of fish meal or scraps on the market were tested for the content of ether and acetone extract, and the iodine value of these extracts.

The results of the above experiment are summarized as follows :—

1. The content of ether extract in sardine meal decreased more or less during storage. The decrease was half when the sardine meal was exposed to the air during a storage period of six monthes. The same decrease was attained by heat of 80~100°C for a few days. The iodine value of such extract decreased in a marked degree.

2. Sardine and herring oil increased in weight during the heating process. But the solubility in ether was decreased.

3. Sardine meal, in which the ether extract content decreased during storage, recovered much of this content again, when it was extracted after HCl hydrolysis, although it recovered less after pepsin digestion.

4. The above experiments prove that the oil in fish meal is oxydized by oxygen in the air during storage or heating and decreases its solubility by air.

5. Acetone extracts much more oxydized oil than does ether. Benzol has intermediate solubility.

6. Fish meal or scrap on the market are almost always more or less oxydized and the ether extract of the more oxydized meal is less in quantity.

Über die Gärungsmikroorganismen in Awamori-Bereitung (II)

Saccharomyces-Arten. (S. 1163~1184): von Ryôdi NAKAZAWA und Mituo SIMO. (The Department of Industry, Government Reserch Institute, Taiwan, Japan; Received Aug. 20, 1936.)

Man hatte bisher geglaubt, dass der wichtigste Hefepilz der Awamori-Gärung nur die eine Art, nämlich *Saccharomyces Awamori Inui* ist, aber nach dieser Untersuchung ist erkannt worden, dass mindestens *Saccharomyces* von

6 voreinander verschiedenen Typen dabei mitwirken.

On the Koji-amylase. (pp. 1185~1202): By Yuzo TOKUOKA. (Tsune-kichi Okura Brewery, Received September 24, 1936.)

Part I.—The Effect of Brewing Water on the Extraction of Koji-amylase.

It was found that the extraction of amylase from Koji (*Aspergillus oryzae* grown on steamed rice) was very limited when distilled water was being used.

A remarkable effect of neutral salts on the extraction of Koji-amylase was verified with NaCl solution: greater amount (nearly four times) of amylase was extracted with 0.1~1.0% NaCl solution, and the extraction was again increased (nearly twice) with such a dilute solution as 0.02% NaCl, when these were compared with distilled water.

Experiments were carried out with various kinds of brewing water, and it was pointed out that the extraction of amylase was found to be coincident to their amounts of total evaporation residues, so that the action of Miya-water (famous brewing water for Saké manufacture) was suggested, in a great measure, to be due its effective power for the extraction of Koji-amylase.

Part II.—The Fluctuation of Amylase during the Fermentation of Saké-moromi (Saké-mash), and the Adsorption of Amylase by Steamed Rice.

In the previous paper (see Part I), it was concluded that NaCl would produce a decisive effect on the extraction of Koji-amylase, therefore the total amount of amylase of Saké-mash would easily be calculated when the diastatic power of the filtrate of the mash after being added NaCl, was determined.

Experiments were carried out, in the present paper, with Saké-mashes at various stages of fermentation.

In the early stages of fermentation (2~5 days), any remarkable change in the total amount of amylase of Saké-mash was not observed, however, the destruction of amylase was gradually taken place after the stage of rocky head (6~7 days) until the end of fermentation (21 days) where the destruction of amylase was reached to 59% of the total amount.

As was already discussed by several authors, the fluctuation of amylase in the direct filtrate of Saké-mash without being added NaCl, was very irregular, since in the early stage of fermentation (2~3 days) the least amount (19% of the total amount) of amylase was found in the filtrate, while the maximum amount (nearly 69% of the total amount) of amylase was found at the stages of between rocky and yeasty heads (6~12 days), and the amount

of amylase was gradually decreased during the further fermentation until it was found to be 26% of the total amount of amylase at the end of the fermentation.

This peculiar fluctuation in the early stage of fermentation suggested that steamed rice existed in Saké-mash would adsorp amylase.

With Taka-diastase solution, it was ascertained that steamed rice revealed a remarkable adsorption of amylase, while any noticeable amount of adsorption of amylase was not observed with rice starch or oryzzenin.

It is probable that larger amount of amylase was found in the spent of Saké, since the adsorption of amylase by steamed rice was accelerated in the presence of alcohol, while any remarkable effect in the degree of adsorption was not observed by pH values (4.0~6.2).

Part III—Effect of Solvents upon the Amount of Amylase extracted from Saké-Koji of various Periods of Cultivation.

For the solvents, distilled water and 1% NaCl solution were chosen in the experiments.

The amount of amylase extracted from Koji was always found to be increased according to the periods of cultivation of Saké-koji, however, the dextrinising and the saccharifying activities or the rates of increase of the diastatic powers of the extracts differed greatly between these solvents.

With salt solution, the rate of increase of both diastatic activities was found to be quite identical ; both the dextrinising and the saccharifying activities of the extracts were gradually increased according to the periods of cultivation of Saké-koji, until these activities reached three times of the youngest stage of Koji during the last 16 hours' cultivation, therefore, the ratio of these activities was always constant at a given stage of cultivation.

With distilled water, the amount of amylase extracted from Koji was always found to be inferior to that of extracts with salt solution as was already mentioned (see Part I and II), however, the rate of increase of the diastatic powers was much superior to the latter case and the different rate of increase in the two activities of amylase was pointed out at the various stages of cultivation of Saké-koji.

The greatest increase was found with the dextrinising activity which was accelerated more than eleven times during the last 16 hours' cultivation, while the saccharifing activity was increased nearly 6 times during these cultivation, therefore the ratio $\frac{\text{dextrinising power}}{\text{saccharifying power}}$ was doubled during these periods.

These experimental results suggested that amylase of Saké-koji would partly exist in soluble-state and the other parts would be adsorped by starch

of the steamed rice with which amylase could not be extracted by distilled water, and maltase of Saké-koji would be easily extracted by water.

It is interesting to note that any remarkable acceleration of salt water in the extraction of amylase from Soya-koji which contained very trace of starchy materials, was not observed.

It is quite probable that the ratio $\frac{\text{dextrinising power}}{\text{saccharifying power}}$ in the water extracts was increased during the periods of cultivation, since maltase would always be easily extracted which revealed a great influence upon the saccharifying activity, while the extraction of amylase would be limited to its soluble state whose amount would be increased during the cultivation of Saké-koji owing to the production of amylase and the decomposition of starch by the mould.

It is again probable that any noticeable changes in the ratio dextrinising power/saccharifying power in the extracts with salt water was not observed, when salt solution would completely extract both the amylase and the maltase of Saké-koji.

Part IV.—Attempts to the Preparation of Taka-amylase free from Maltase.

It has long been believed that Koji-amylase revealed a special nature of decomposing starch into glucose, since any successful results for the separation of Koji-amylase into their components; maltase and ordinary amylase which would decompose starch into maltose, although several attempts were already made by many authors.

It was found in the previous paper (Part III) that the ratio of dextrinising and saccharifying power in the water extracts of Saké-koji would be altered according to the period of cultivation and suggested that these variations would be due to the different solubilities of maltase and of the amylase adsorbed by starch.

If this suggestion would be probable, the preparation of Taka-amylase free from maltase could easily be obtained, since the amylase adsorbed by starch would be extracted by salt solution as was already ascertained (Part I & II).

In the present paper, experiments were carried out with different kinds of Saké-koji in the following manner: Koji was extracted more than ten times with distilled water in the object of washing out maltase as far as possible, and then extracted the residual amylase with 0.5% NaCl solution. After being condensed the sodium chloride solution, amylase was precipitated by alcohol.

It was found that preparation of the Taka-amylase was almost free from maltase, since the amount of maltase in the preparation was calculated low-

er than 1/140 of that of the aqueous preparation when the preparations were compared to reveal the same dextrinising activity.

The product of hydrolysis of starch by the Taka-amylase preparation was observed to be maltose, and any evidence of the glucose could not be obtained since the hydrolysate did not reduce Barford reagent and no glucosazone was obtained from the hydrolysate.

Studies on Vitamin C. XVIII.—The Effect of Light upon the Production of Vitamin C. (pp. 1203~1210): By Tomiji MATSUOKA. (The Tokyo Agricultural Colledge, Received Sept. 25, 1936.)

According to Fürst, Chick and Hume, Heller, Kucera, it is an established fact that vitamin C is synthesized by the process of germination. The writer, also reported that germination of various seeds in the sun light produced a notable amount of vitamin C, and showed that although, light was not absolutely necessary factor for production of vitamin C, but its content was affected by the light, and the difference of content was more clearly defined in sprouts germinated for a long time.

In the 17th report, the writer stated that barley produced antiscorbutic vitamin even in the dark, but existence of light had some influence upon the production, for vitamin C was remarkably increased in barley cultivated in the sun light.

The writer examined the effect of light upon the production of vitamin C with barley germinated, radish grown for 35 days and Unshu orange. Results obtained were as follows.

(1) In sprouts no manured during germination was effected by the light, the longer the seeds germinated, the greater the difference of content.

(2) The production of vitamin C in sprouts manured was also clearly affected by the light. To compare the content of the sprouts germinated in the dark and that of the sprouts germinated in the sun light, the former was only about 1/4 of the latter.

(3) One part of barley were covered to grow in the dark field, until the barley not covered extends to the length of 4 feet, to see whether there is an influence of light upon the production of vitamin C, according to the result, it is an established fact that the existence of light influences upon the production of vitamin C, for, the production was about quadruple in the light as much as in the dark.

(4) The radish germinated were grown for 15 days in the sun light, then, one part of them covered for 20 days to grow in the dark, the other part were grown in the sun light. The content of vitamin C of the radish covered was about 1/5 of the other.

(5) Vitamin C content of uncovered orange was twice as much as that of covered one, therefore, the effect of light was very little.

(6) The production of vitamin C during life of plant is affected by the light.

(7) Light is not the absolute factor for the production of vitamin C during life of plant, for it was produced even in the dark, but the existence of light has some influence upon the production.

On the Derivative of Vitamin-D and Several Sterins. (pp. 1211 ~1216): By Midzuho SUMI. (The Institute of physical and chemical Research, Tokyo, Japan, Received Oct. 5, 1936.)

The vitamin-D crystals were isolated by saponifying the vitamin-D β -naphtoate obtained from ultra-violet irradiated ergosterol.

Also the crystalline derivatives of cholesterol, sitosterin and ergosterin, such as β -naphtoate and β -anthrachinoncarbonic acid ester were isolated.

Results were as follows:

(1) Vitamin-D naphtoate: needles, mp 132°, $(\alpha)_D^{20} = +149.97$.

Vitamin-D: needles or prisms, mp 115°~116°, Absorption spectrum max. 265 m μ .

(2) β -Naphtoate. β -Anthrachinoncarbonic acid ester.

(a) Cholesterol: needles, mp 163°. yellow needles or prisms, mp 170.

$(\alpha)_D^{20} = 0$ (or above 250°), $(\alpha)_D^{20} = 0$.

(b) Sitosterin: needles, mp 190°. yellow needles, mp 189° (or 253°).

$(\alpha)_D^{20} = +2.5$ $(\alpha)_D^{20} = -1.3$.

(c) Ergosterin: needles, mp 175°. yellow needles, mp 195° (200°).

The antirachitic potency of vitamin D crystal was determined by biological test. The administration of 0.025 γ for rat daily was enough for the healing of rickets, i.e. the activity of vitamin D crystal corresponds to 40000 International Units per mg.

On the Optical Properties of the Fermentation Lactic Acids.

Part. V—The action of acetone-butyl alcohol producing organism upon optically active lactic acids. (pp. 1217~1220): By Hideo KATAGIRI and Kakuo KITAHARA. (Agr. Chemical Laboratory, Kyoto Imperial University, Received Oct. 6, 1936.)

In order to ascertain the reason why inactive lactic acid would be obtained in the mixed cultures of an optically active lactic acid producing organism and an acetone-butyl alcohol producing organism, series of experiments were carried out with Clostridium acetobutylicum.

When Cl. acetobutylicum was cultured with *l*-former (*Leuconostoc mesenteroides* var. *Sake*), all the lactic acid obtained from malt extract was found to be inactive.

Very remarkable racemisation of the lactic acids previously added to the malt extract was observed when Cl. acetobutylicum was inoculated to the cultural solution.

It was verified that *Cl. acetobutylicum* revealed a remarkable racemisation of active lactic acids even in its resting state.

Thus it was concluded that the special effect of *Cl. acetobutylicum* in causing fermentation lactic acid inactive, would be again due to the action of racemiase of the bacteria as was discussed in the previous paper (see Part IV) with *dl*-formers.

Studies on the Composition of the dried Meat of the Sea-ear and the Glycogenase of the fresh Sea-ear (*Haliotis gigantea* Gm). (pp. 1221~1226): By Kisuke KONDO and Sakae SHINANO. (Laboratory of the Nutritional Chemistry, Kyoto Imp. University, Received Oct. 7, 1936.)

(1) Two kinds of the dried meats of sea-ear were analysed with the results that the water-content was 35~38% and the principal compositions of the dried matter were protein and glycogen. And it was explained that the amount of the latter should be fluctuated by the many factors—the living place, season, age etc—as well as the amount of fat in the fishes- or the higher animals-bodies.

(2) The meat was hydrolysed and the nitrogen-distribution was determined by the routine method. According this result the meat of sea-ear could not be estimated that it was so highly nutritious as considered commonly. If it were, it would be caused by the fine texture of the meat, the taste from the glutamic acid and the large amount of glycogen.

(3) The glycogen was isolated and purified from the dried meat with the yield of about 10%.

(4) The occurrence of the glycogenase in the fresh meat and intestines of sea-ear was confirmed. The activity of this enzyme decreased during conservation after it was isolated, but the decrease did not continue. The optimum paH value for the activity of this enzyme was near paH=5.

(1936, Sept. 18)

Biochemical Investigation of Mosaic Disease of Tobacco Plants. I.—Influence of the Concentrations of Hydrogen Ion and of Nutrients in Culture Solution. (pp. 1227~1231): By Yuzuru OKUDA and Hiroshi SUTOH. (Department of Agriculture, Kyushu Imp. University, Received Oct. 7, 1936.)

Biochemical Investigation of Mosaic Disease of Tobacco Plants. II.—Distribution of Substances in both Healthy and Diseased Leaves. (pp. 1232~1236): By Yuzuru OKUDA, Shinobu SHIGEMATHU and Minoru HANADA. (Department of Agriculture, Kyushu Imp. University, Fukuoka, Received Oct. 7, 1936.)